

PREPARATION AND CHARACTERIZATION OF PVA AND CARRAGEENAN BASED HYDROGELS FOR BIOMEDICAL PURPOSES

**Thesis submitted to
National Institute of Technology, Rourkela
For the partial fulfillment
Of the
Master degree in Life Science**

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CERTIFICATE

This is to certify that the thesis entitled "*Preparation and characterization of Carrageenan and PVA based hydrogels for biomedical application*" Submitted to National Institute of Technology, Rourkela for the partial fulfillment of the Master degree in Life science is a faithful record of bonafide and original research work carried out by **Ms. Arpita Nanda** under my supervision and guidance. The results embodied in this thesis are new and have not been submitted to any other university or institution for award of any degree or diploma.

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DECLARATION

I hereby declare the thesis entitled "*Preparation and characterization of PVA and carrageenan based Hydrogels for biomedical applications*", submitted to the Department of Life Science, National Institute of Technology, Rourkela for the partial fulfilment of the Master Degree in Life Science is a faithful record of bonafide research work carried out by me under the guidance and supervision of Dr. Bismita Nayak, Assistant Professor, Department of Life Science, National Institute of Technology, Rourkela. No part of this thesis has been submitted by any other research persons or any students.

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Place: Rourkela
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ABSTRACT

Hydrogels are three-dimensional, cross- linked networks of water-soluble polymers. They can be made from nearly any water-soluble polymer, covering a wide range of chemical compositions and bulk physical properties. These are highly porous . For our preparation of hydrogels we have used 10% PVA with 2% Carrageenan . cross linker was added for the preparation of gel . Characterizations were done Physiochemical characterization and other characterization . Under physiochemical characterization we have done SEM , XRD , MICROSCOPY, ELECTRICAL AND MECHANICAL . And under other characterization we have done Hemocompatibility , Drug release , Swelling, Antimicrobial and MTT Assay. The above characterisation of the hydrogels showed that the hydrogels can be used for a control drug delivery system. It can be used for biomedical applications. We found that CP 6 shows the better result as comparable to the rest of the concentrations as it shows the high hemocompatibility and antimicrobial activity. It also shows good swelling ability and uniform drug release profile

INTRODUCTION

Hydrogels are three-dimensional, cross-linked networks of water-soluble polymers. They can be made from nearly any water-soluble polymer, covering a wide range of chemical compositions and bulk physical properties. Hydrogels are commonly used in clinical practice and experimental medicine for a wide range of applications, including tissue engineering and regenerative medicine, diagnostics, cellular immobilization, separation of bio-molecules or cells, and barrier materials to regulate biological adhesions. The unique physical properties of hydrogels have provoked a particular interest in their use in drug delivery applications. Their highly porous structure can easily be tuned by controlling the density of cross-links in the gel matrix and the affinity of the hydrogels for the aqueous environment in which they are swollen. If water is removed from these swollen biomaterials, they are called xerogels, which are the dried hydrogels. These gels may be charged or non-charged depending on the nature of functional groups present in their structure. The charged hydrogels usually exhibit changes in swelling upon variations in pH, and it is known that they can undergo changes in shape when exposed to an electric field [3]. The enormous attention is the parameters control by which the degradation characteristics can be adapted. As the gels are used, this is of extreme importance that the hydrogels have excellent biocompatibility and degradation products produced have a low toxic potential. Hydrogels hold excellent biocompatibility. Its water loving surface has fewer propensities for cells and proteins to stick to these surfaces. Furthermore, the elastic and soft nature of gels minimizes irritability to the neighboring tissues (Park and Park, 1996, Smetana, 1993, Anderson and Langone, 1999 and Anderson, 1994). The properties of degradation products produced may be modified by the proper and rational selection of the starting materials of hydrogel. Depending on the type of cross-linking, hydrogels can be divided into two classes: (i) chemically cross-linked hydrogels, and (ii) physically cross-linked hydrogels. Chemical hydrogels are formed by covalent networks and do not dissolve in water without breakage of covalent bonds [12], [13], [14] and [15]. Physical hydrogels are, however, formed by dynamic cross-linking of synthetic or natural building blocks based on non-covalent interactions such as hydrophobic and electrostatic interaction or hydrogen-bridges [16], [17] and [18]. The hydrophilic and mostly inert nature of hydrogels often leads to minimized non-specific interaction with proteins and cells which makes hydrogels ideal candidates for numerous bio-

related applications [19] and [20]. For most medical application, the novel engineering of hydrogels for drug delivery require dividing them to biodegradable hydrogels which are favoured over non-degradable hydrogels since they degrade in clinically relevant time scale [8], smart hydrogel or stimuli-sensitive hydrogel that respond to environmental changes, such as temperature, pH, light, and specific molecules such as glucose [9] and finally biomimetic hydrogels which are relatively inert polymer chains can be tailored with the selected biological moieties to yield bioactive hydrogels [10].

PVA is a water-soluble synthetic polymer. It has the idealized formula $[\text{CH}_2\text{CH}(\text{OH})]_n$. It is used in papermaking, textiles, and a variety of coatings. It is white (colourless) and odorless. It is sometimes supplied as beads or as solutions in water.[1] Polyvinyl Alcohol (PVA) is an environmental friendly and water soluble synthetic polymer with excellent film forming property, and emulsifying properties and outstanding resistance to oil, grease, and solvents. It has been extensively used in adhesive, in textile warp sizing and finishing, in paper size and coating, in the manufacturing of PVAc emulsion, in the suspension polymerization of PVC, and as binder for ceramics, foundry cores and several of pigment. PVA is manufactured by polymerization of vinyl acetate monomer, followed by hydrolysis of the polymerization of vinyl acetate monomer, followed by hydrolysis of the polyvinyl acetate. Since the properties on which technical application of PVA depend are primarily its molecular weight and degree of hydrolysis. The amount of hydroxylation determines the physical characteristics, chemical properties, and mechanical properties of the PVA.¹ The resulting PVA polymer is highly soluble in water but resistant to most organic solvents. The higher the degree of hydroxylation and polymerization of the PVA, the lower the solubility in water and the more difficult it is to crystallize.² Due to its water solubility, PVA needs to be crosslinked to form hydrogels for use in several applications. The crosslinks, either physical or chemical, provide the structural stability the hydrogel needs after it swells in the presence of water or biological fluids.³ The degree of crosslinking dictates the amount of fluid uptake, and thus the physical, chemical, and diffusional properties of the polymer, and ultimately its biological properties. PVA's resistance against organic solvents and aqueous solubility makes it adaptable for many applications.^{1, 2} PVA is commonly used in the textile industries, for paper products manufacturing, in the food packaging industry, and as medical devices. PVA is used as an industrial and commercial product due to its low environmental impact, which includes its high chemical resistance, aqueous solubility, and

biodegradability. FDA has approved PVA to be in close contact with food products; in fact, PVA films exhibit excellent barrier properties for food packaging systems. In medical devices, PVA is used as a biomaterial due to its biocompatible, nontoxic, noncarcinogenic, swelling properties, and bioadhesive characteristics.

Carrageenan has the ability to form strong gels with certain salts or other gums and its ability to interact with certain dairy proteins. Carrageenan is mainly used in the food industry with some applications in the toiletries industry. Industrial applications of carrageenan are rare. Carrageenan has three basic forms:-

Lambda Carrageenan

Iota Carrageenan

Kappa Carrageenan

Carrageenan is a generic name for a family of gel-forming and viscosifying polysaccharides, which are obtained by extraction from certain species of red seaweeds (Table 1). Carrageenan is derived from a number of seaweeds of the class Rhodophyceae. Carrageenans are composed of a linear galactose backbone with a varying degree of sulfatation (between 15% and 40%). Different carrageenan types differ in composition and conformation resulting in a wide range of rheological and functional properties. Carrageenans are used in a wide variety of commercial applications as gelling, thickening, and stabilizing agent in especially food products, such as frozen desserts, chocolate-milk, cottage cheese, whipped cream, instant products, yogurt, jellies, pet foods, sauces, and so forth.

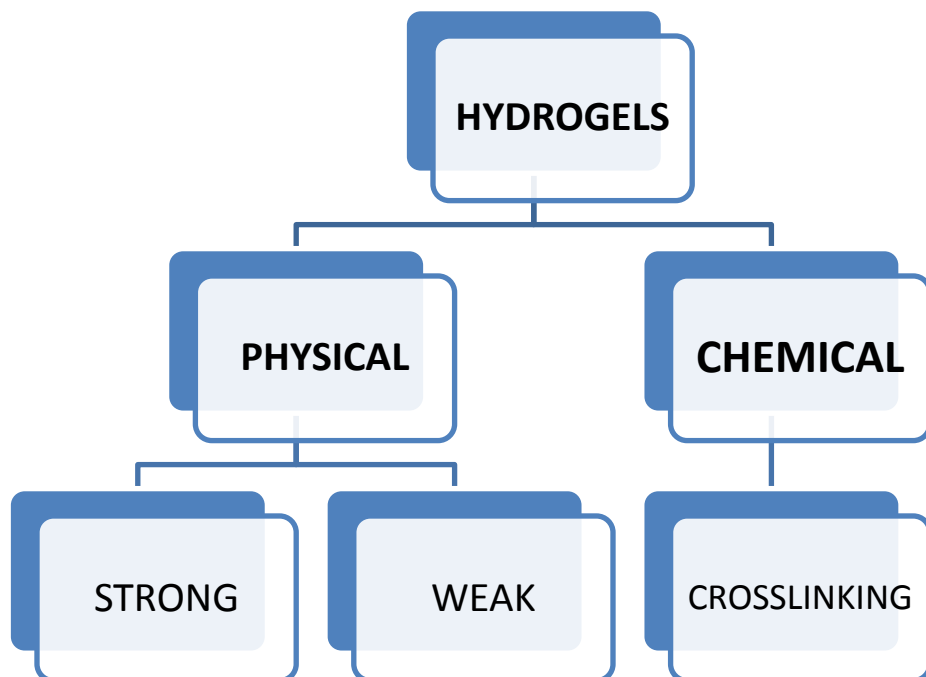
Ciprofloxacin (INN) is an antibiotic that can treat a number of bacterial infections. It is a second-generation fluoroquinolone.[2][3] Its spectrum of activity includes most strains of bacterial pathogens responsible for respiratory, urinary tract, gastrointestinal, and abdominal infections, including Gram-negative (*Escherichia coli*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Moraxella catarrhalis*, *Proteus mirabilis*, and *Pseudomonas aeruginosa*), and Gram-positive (methicillin-sensitive, but not methicillin-resistant *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, and *Streptococcus pyogenes*) bacterial pathogens. Ciprofloxacin and other fluoroquinolones are valued for this broad spectrum of activity, excellent tissue penetration, and for their availability

in both oral and intravenous formulations.[4][page needed] Ciprofloxacin is used alone or in combination with other antibacterial drugs in the empiric treatment of infections for which the bacterial pathogen has not been identified, including urinary tract infections[5][6] and abdominal infections[7] among others. It can also treat infections caused by specific pathogens known to be sensitive. Ciprofloxacin is the most widely used of the second-generation quinolone antibiotics that came into clinical use in the late 1980s and early 1990s.[8][9] In 2010, over 20 million outpatient prescriptions were written for ciprofloxacin, making it the 35th-most commonly prescribed drug, and the 5th-most commonly prescribed antibacterial, in the US.[10] Ciprofloxacin was discovered and developed by Bayer A.G. and subsequently approved by the US Food and Drug Administration (FDA) in 1987. Ciprofloxacin has 12 FDA-approved human uses and other veterinary uses, but it is often used for unapproved uses.

REVIEW OF LITERATURE

Hydrogels are polymer networks extensively swollen with water. These are three dimensional crosslinked architectures with proven multifaceted applications. The hydrogels are known to have good water interaction and swell when exposed to aqueous solution [1]. When swelled they become soft and rubbery properties which could be tailored to apply them into different field of pharmacy, pharmaceuticals and biomedical engineering [2]. Natural hydrogels were gradually replaced by synthetic hydrogels which has long service life, high capacity of water absorption, and high gel strength. Fortunately, synthetic polymers usually have well-defined structures that can be modified to yield tailor able degradability and functionality. Hydrogels can be synthesized from purely synthetic components. Also, it is stable in the conditions of sharp and strong fluctuations of temperatures [3]. Hydrogels have been defined as two- or multi-component systems consisting of a three-dimensional network of polymer chains and water that fills the space between macromolecules. Depending on the properties of the polymer (polymers) used, as well as on the nature and density of the network joints, such structures in an equilibrium can contain various amounts of water; typically in the swollen state, the mass fraction of water in a hydrogel is much higher than the mass fraction of polymer. Hydrogels may exhibit drastic volume changes in response to specific external stimuli, such as the temperature, solvent quality, pH, electric field, etc. [5]. Depending on the design of the hydrogel matrices, this volume change may occur continuously over a range of stimulus level, or, discontinuously at a critical stimulus level. The volume transition behaviours of hydrogels received considerable interest in the last three decades and large parts of the work have been collected in different reviews [7]. Gelation refers to the linking of macromolecular chains together which initially leads to progressively larger branched yet soluble polymers depending on the structure and conformation of the starting material. The mixture of such polydisperse soluble branched polymer is called 'sol'. Hydrogels can take place either by physical linking (physical gelation) or by chemical linking (chemical gelation). Physical gels can be sub categorised as strong physical gels and weak gels. Strong physical gel has strong physical bonds between polymer chains and is effectively permanent at a given set of experimental conditions [4]. Different types of gelation mechanism are summarised in Figure 1. Gelation can take place either by physical linking (physical gelation) or by chemical linking (chemical gelation). Physical gels can be sub categorised as strong physical gels and weak gels. Strong physical gel has strong physical bonds between polymer chains and is effectively permanent. Hence, strong physical gels are analogous to chemical gels. Examples of strong physical bonds are lamellar microcrystals, glassy nodules or double and triple helices. Weak physical gels have reversible links formed from temporary associations between chains [5]. Weak physical bonds are hydrogen bond, block copolymer micelles, and ionic associations. On the other hand, chemical gelation involves formation of covalent bonds and always results in a strong gel. Hydrogels based on natural or synthetic polymers have been of great interest

regarding cell encapsulation [6], For the past decade, such hydrogels have become especially attractive as matrices for regenerating and repairing a wide variety of tissues and organs



CLASSIFICATION OF HYDROGELS:-

Depending on their method of preparation, ionic charge, or physical structure features, hydrogels may be classified in several categories. Based on the method of preparation, they may be (i) homopolymer hydrogels, (ii) copolymer hydrogels, (iii) multipolymer hydrogels, or (iv) interpenetrating polymeric hydrogels. They are broadly classified into Permanent / chemical gel: they are called 'permanent' or 'chemical' gels when they are covalently cross-linked (replacing hydrogen bond by a stronger and stable covalent bonds) networks (Hennink & Nostrum, 2002). They attain an equilibrium swelling state which depends on the polymer-water interaction parameter and the crosslink density (Rosiak & Yoshii, 1999). Reversible / physical gel: they are called 'reversible' or 'physical' gels when the networks are held together by molecular entanglements, and / or secondary forces including ionic, hydrogen bonding or hydrophobic interactions. In physically cross-linked gels, dissolution is prevented by physical interactions, which exist between different polymer chains (Hennink & Nostrum, 2002). All of these interactions are reversible, and can be disrupted by changes in physical conditions or application of stress [6].

- Homopolymeric hydrogels are referred to polymer network derived from a single species of monomer, which is a basic structural unit comprising of any polymer network [8].

Homopolymers may have cross-linked skeletal structure depending on the nature of the monomer and polymerization technique.

- Copolymeric hydrogels are comprised of two or more different monomer species with at least one hydrophilic component, arranged in a random, block or alternating configuration along the chain of the polymer network [9].
- Multipolymer Interpenetrating polymeric hydrogel (IPN), an important class of hydrogels, is made of two independent cross-linked synthetic and/or natural polymer component, contained in a network form. In semi-IPN hydrogel, one component is a cross-linked polymer and other component is a non-cross-linked polymer [10] and [11].

CLASSIFICATION BASED ON CONFIGURATION:-

The classification of hydrogels depends on their physical structure and chemical composition can be classified as follows:

- (a) Amorphous (non-crystalline).
- (b) Semicrystalline: A complex mixture of amorphous and crystalline phases.
- (c) Crystalline.

CLASSIFICATION BASED ON TYPE OF CROSSLINKING:-

Hydrogels appears as matrix, films or microsphere depends on the technique of polymerization involved in the preparation process.

CLASSIFICATION ACCORDING TO NETWORK ELECTRICAL CHARGE :-

Hydrogels may be categorized into four groups on the basis of presence or absence of electrical charge located on the cross-linked chains:

- (a) Nonionic (neutral).
- (b) Ionic (including anionic or cationic).
- (c) Amphoteric electrolyte (ampholytic) containing both acidic and basic groups.
- (d) Zwitterionic (polybetaines) containing both anionic and cationic groups in each structural repeating unit.

Hydrogel-forming natural polymers include proteins such as collagen and gelatine and polysaccharides such as starch, alginate, and agarose. Synthetic polymers that form hydrogels are traditionally prepared using chemical polymerization methods.

CHARACTERISTICS OF HYDROGELS :-

The water holding capacity and permeability are the most important characteristic features of a hydrogel. The polar hydrophilic groups are the first to be hydrated upon contact with water which leads to the formation of primary bound water. As a result the network swells and exposes the hydrophobic groups which are also capable of interacting with the water molecules. This leads to the formation of hydrophobically-bound water, also called 'secondary bound water'. Primary and secondary bound water are often combined and called 'total bound water'. The network will absorb additional water, due to the osmotic driving force of the network chains towards infinite dilution [11] This additional swelling is opposed by the covalent or physical cross-links, leading to an elastic network retraction force. Thus, the hydrogel will reach an equilibrium swelling level. The additional absorbed water is called 'free water' or 'bulk water', and assumed to fill the space between the network chains, and/or the centre of larger pores, macropores, or voids. Depending on the nature and composition of the hydrogel the next step is the disintegration and/or dissolution if the network chain or cross-links are degradable. Biodegradable hydrogels, containing labile bonds, are therefore advantageous in applications such as tissue engineering, wound healing and drug delivery. These bonds present can be either polymer backbone or in the cross-links used to prepare the hydrogel. [10]. Biocompatibility is the third most important characteristic property required by the hydrogel. Biocompatibility calls for compatibility with the immune system of the hydrogel and its degradation products formed, which also should not be toxic. Ideally they should be metabolised into harmless products or can be excreted by the renal filtration process. Generally, hydrogels possess a good biocompatibility since their hydrophilic surface has a low interfacial free energy when in contact with body fluids, which results in a low tendency for proteins and cells to adhere to these surfaces. Moreover, the soft and rubbery nature of hydrogels minimises irritation to surrounding tissue (Anderson & Langone, 1999; Smetana, 1993). The cross-links between the different polymer chains results in viscoelastic and sometimes pure elastic behaviour and give a gel its structure (hardness), elasticity and contribute to stickiness. Hydrogels, due to their significant water content possess a degree of flexibility similar to natural tissue. It is possible to change the chemistry of the hydrogel by controlling their polarity, surface properties, mechanical properties, and swelling behaviour.

USES OF HYDROGELS:-

Common uses for hydrogels include:

- Scaffolds in tissue engineering. When used as scaffolds, hydrogels may contain human cells to repair tissue. They mimic 3D microenvironment of cells.[10]
- Hydrogel coated wells were used for cell cultures.

- Environmentally sensitive hydrogels (also known as 'Smart Gels' or 'Intelligent Gels'). These hydrogels have the ability to sense changes of pH, temperature, or the concentration of metabolite and release their load as result of such a change.
- Sustained- released drug delivery system.
- It provides absorption , desloughing and debriding of necrotic fibrotic tissue.
- Used in disposable diapers where they absorb urine or in sanitary napkins.
- Used for contact lenses .
- Rectal drug delivery and diagnosis.
- For breast implants
- Glue
- Granules for holding soil moisture in arid areas.
- Dressings for healing of burn.
- Reservoirs in topical drug delivery ; particularly ionic drugs , delivered by iontophoresi.

Natural hydrogel materials are being invested for tissue engineering, these materials include agarose ,methylcellulose ,hyaluronan , and other naturally derived polymers.

There are various other types of gels which include :-

ORGANOGELES:-

An organogel is a non-crystalline, non-glassy thermoreversible (thermoplastic) solid material composed of a liquid organic phase entrapped in a three-dimensionally cross-linked network. The liquid can be, for example, an organic solvent, mineral oil, or vegetable oil. The solubility and particle dimensions of the structurant are important characteristics for the elastic properties and firmness of the organogel. Often, these systems are based on self-assembly of the structurant molecules.[13][14]Organogels have potential for use in a number of applications, such as in pharmaceuticals,[15] cosmetics, art conservation,[16] and food.[17] An example of formation of an undesired thermoreversible network is the occurrence of wax crystallization in petroleum.[18]

XEROGELS:-

A xerogel is a solid formed from a gel by drying with unhindered shrinkage. Xerogels usually retain high porosity (15–50%) and enormous surface area (150–900 m²/g), along with very small pore size (1–10 nm). When solvent removal occurs under supercritical conditions, the network does not shrink and a highly porous, low-density material known as an aerogel is produced. Heat treatment of a xerogel at elevated temperature produces viscous sintering (shrinkage of the xerogel due to a small amount of viscous flow) and effectively transforms the porous gel into a dense glass.

NANOCOMPOSITE HYDROGELS:-

Nanocomposite hydrogels are also known as hybrid hydrogels, can be defined as highly hydrated polymeric networks, either physically or covalently crosslinked with each other and/or with nanoparticles or nanostructures. Nanocomposite hydrogels can mimic native tissue properties, structure and microenvironment due to their hydrated and interconnected porous structure. A wide range of nanoparticles, such as carbon-based, polymeric, ceramic, and metallic nanomaterials can be incorporated within the hydrogel structure to obtain nanocomposites with tailored functionality. Nanocomposite hydrogels can be engineered to possess superior physical, chemical, electrical, and biological properties.[19]

PROPERTIES OF HYDROGELS:--

Many gels display thixotropy – they become fluid when agitated, but resolidify when resting. In general, gels are apparently solid, jelly-like materials. By replacing the liquid with gas it is possible to prepare aerogels, materials with exceptional properties including very low density, high specific surface areas, and excellent thermal insulation properties.

APPLICATIONS:-

Biomedical Applications:-

Their biocompatibility allows them to be considered for medical applications, whereas their hydrophilicity can impart desirable release characteristics to controlled and sustained release formulations. Hydrogels exhibit properties that make them desirable candidates for biocompatible and blood-compatible biomaterials (Merrill et al., 1987).

One of the most earliest use of hydrogels was that they are used in contact lenses because of their good mechanical stability and high oxygen permeability.

Other potential applications include:-

- Artificial tendon materials
- Wound healing bioadhesives

- Artificial kidney membranes
- Artificial skin
- Maxillofacial
- Vocal cord replacement materials

Pharmaceutical Applications:-

Pharmaceutical applications have become very much popular, these systems include equilibrium swollen hydrogels. The category of solvent-activated, matrix-type, controlled-release devices comprises two important types of systems: swellable and swelling-controlled devices. In general, a system prepared by incorporating a drug into a hydrophilic, glassy polymer can be swollen when brought in contact with water or simulant of biological fluids. This swelling process may or may not be the controlling mechanism for diffusional release, depending on the relative rate. In swelling-controlled release systems, the bioactive agent is dispersed into the polymer to form nonporous films, disks, or spheres. Upon contact with an aqueous dissolution medium, a distinct front (interface) is observed that corresponds to the water penetration front into the polymer and separates the glassy from the rubbery (gel-like) state of the material. Under these conditions, the macromolecular relaxations of the polymer influence the diffusion mechanism of the drug through the rubbery states of the macromolecular relaxation of the polymer and drug diffusion from the gel. This water uptake can lead to considerable swelling of the polymer with a thickness that depends on time. The swelling process proceeds toward equilibrium at a rate determined by the water activity in the system and the structure of the polymer. If the polymer is cross-linked or if it is of sufficiently high molecular weight (so that chain entanglements can maintain structural integrity), the equilibrium state is a water-swollen gel. The equilibrium water content of such hydrogels can vary from 30% to 90%. If the dry hydrogel contains a water-soluble drug, the drug is essentially immobile in the glassy matrix, but begins to diffuse out as the polymer swells with water. Drug release thus depends on two simultaneous rate processes: water migration into the device and drug diffusion outward through the swollen gel. Since some water uptake must occur before the drug can be released, the initial burst effect frequently observed in matrix devices is moderated, although it may still be present. The continued swelling of the matrix causes the drug to diffuse increasingly easily, ameliorating the slow tailing off of the release curve.

ADVANTAGES OF HYDROGELS:-

- Hydrogels possess a degree of flexibility very similar to natural tissue due to their significant water content.
- Entrapment of microbial cells within hydrogels beads has the advantages of low toxicity.
- Hydrogels have good transport properties.

- Hydrogels are biocompatible.
- Hydrogels can be injected.
- These are very easy to modify.

For our experiment we have used PVA and Carrageenan for hydrogel preparation.

PVA (Poly – Vinyl Alcohol):-

Poly(vinyl alcohol) (PVOH, PVA, or PVAI) is a water-soluble synthetic polymer. It has the idealized formula $[\text{CH}_2\text{CH}(\text{OH})]_n$. It is used in papermaking, textiles, and a variety of coatings. It is white (colourless) and odorless. It is sometimes supplied as beads or as solutions in water.[12]. Another hydrophilic polymer that has received attention is poly(vinyl alcohol) (PVA). This material holds tremendous promise as a biological drug delivery device because it is nontoxic, is hydrophilic, and exhibits good mucoadhesive properties. Polyvinyl acetals: Polyvinyl acetals are prepared by reacting aldehydes with polyvinyl alcohol. Polyvinyl butyral (PVB) and polyvinyl formal (PVF) are examples of this family of polymers. They are prepared from polyvinyl alcohol by reaction with butyraldehyde and formaldehyde, respectively. Preparation of polyvinyl butyral is the largest use for polyvinyl alcohol in the U.S. and Western Europe. Polyvinyl alcohol is used as an emulsion polymerization aid, as protective colloid, to make polyvinyl acetate dispersions. This is the largest market application in China. In Japan its major use is vinylon fiber production.

USES OF POLY-VINYL ALCOHOL:-

- Paper adhesive with boric acid in spiral tube winding and solid board production.
- Thickener, modifier, in polyvinyl acetate glues.
- Textile sizing agent
- Paper coatings, release liner
- As a water-soluble film useful for packaging. An example is the envelope containing laundry detergent in "liqui-tabs".
- Feminine hygiene and adult incontinence products as a biodegradable plastic backing sheet.
- Carbon dioxide barrier in polyethylene terephthalate (PET) bottles.
- As a film used in the water transfer printing process.
- As a form release because materials such as epoxy do not stick to it.
- Movie practical effect and children's play putty or slime when combined with borax
- Used in eye drops (such as artificial tears to treat dry eyes) and hard contact lens solution as a lubricant
- PVA fiber, as reinforcement in concrete.
- Raw material to polyvinyl nitrate (PVN) an ester of nitric acid and polyvinyl alcohol.
- As a surfactant for the formation of polymer encapsulated nanobeads.
- Used in protective chemical-resistant gloves.

- Used as a fixative for specimen collection, especially stool samples.
- When doped with iodine, PVA can be used to polarize light
- As an embolization agent in medical procedures
- Carotid phantoms for use as synthetic vessels in Doppler flow testing
- Used in 3D printing as support structure that can then be dissolved away.

STRUCTURE AND PROPERTIES OF PVA:-

PVA is an atactic material that exhibits crystallinity. In terms of microstructure, it is composed mainly of 1,3-diol linkages $[-CH_2-CH(OH)-CH_2-CH(OH)-]$ but a few percent of 1,2-diols $[-CH_2-CH(OH)-CH(OH)-CH_2-]$ occur, depending on the conditions for the polymerization of the vinyl ester precursor.[1] Polyvinyl alcohol has excellent film forming, emulsifying and adhesive properties. It is also resistant to oil, grease and solvents. It has high tensile strength and flexibility, as well as high oxygen and aroma barrier properties. However these properties are dependent on humidity, in other words, with higher humidity more water is absorbed. The water, which acts as a plasticiser, will then reduce its tensile strength, but increase its elongation and tear strength. PVA has a melting point of 230 °C and 180–190 °C (356–374 degrees Fahrenheit) for the fully hydrolysed and partially hydrolysed grades, respectively. It decomposes rapidly above 200 °C as it can undergo pyrolysis at high temperatures. PVA is close to incompressible.

CARRAGEENAN:-

Carrageenans or carrageenins from Irish *carraigín*, "little rock") are a family of linear sulphated polysaccharides that are extracted from red edible seaweeds. They are widely used in the food industry, for their gelling, thickening, and stabilizing properties. Their main application is in dairy and meat products, due to their strong binding to food proteins. There are three main varieties of carrageenan, which differ in their degree of sulphation. Kappa-carrageenan has one sulphate group per disaccharide. Iota-carrageenan has two sulphates per disaccharide. Lambda carrageenan has three sulphates per disaccharide. Gelatinous extracts of the *Chondrus crispus* (Irish Moss) seaweed have been used as food additives since approximately the 1400s.[1] Carrageenan is a vegetarian and vegan alternative to gelatin in some applications, in some instances it is used to replace gelatin in confectionery. Carrageenan has undergone many long-term dietary studies under defined regulatory conditions en route to its current global regulatory status. While some indicate that carrageenan safely passes through rat GI tracts without adverse effect when it is a dietary ingredient,[2] other animal dietary studies have observed colitis-like disease and tumour promotion.[3] In the late 2000s, some scientists raised concerns about whether the amount of "degraded carrageenan" (poligeenan) in food-grade carrageenan may lead to health problems, leading to a debate in the research literature.[4] It has yet to be determined whether such observations are pertinent to dietary safety considerations. The use of carrageenan

in infant formula, organic or otherwise, is prohibited in the EU for precautionary reasons, but is permitted in other foodstuffs.[5] In the US, it is permitted in organic and non-organic foods, including juices, chocolate milk, and organic infant formula.

PROPERTIES:-

Carrageenans are large, highly flexible molecules that curl forming helical structures. This gives them the ability to form a variety of different gels at room temperature. They are widely used in the food and other industries as thickening and stabilizing agents. All carrageenans are high-molecular-weight polysaccharides made up of repeating galactose units and 3,6 anhydrogalactose (3,6-AG), both sulfated and nonsulfated. The units are joined by alternating α -1,3 and β -1,4 glycosidic linkages. There are three main commercial classes of carrageenan: Kappa forms strong, rigid gels in the presence of potassium ions; it reacts with dairy proteins. It is sourced mainly from *Kappaphycus alvarezii*. [6] Iota forms soft gels in the presence of calcium ions. It is produced mainly from *Eucheuma denticulatum*. [6] Lambda does not gel, and is used to thicken dairy products. The primary differences that influence the properties of kappa, iota, and lambda carrageenan are the number and position of the ester sulfate groups on the repeating galactose units. Higher levels of ester sulfate lower the solubility temperature of the carrageenan and produce lower strength gels, or contribute to gel inhibition (lambda carrageenan). Many red algal species produce different types of carrageenans during their developmental history. For instance, the genus *Gigartina* produces mainly kappa carrageenans during its gametophytic stage, and lambda carrageenans during its sporophytic stage. All are soluble in hot water, but in cold water, only the lambda form (and the sodium salts of the other two) are soluble. When used in food products, carrageenan has the EU additive E-number E407 or E407a when present as "processed eucheuma seaweed". [7] Technically carrageenan is considered a dietary fibre. [8][9] In parts of Scotland and Ireland, where it is known by a variety of local and native names, *Chondrus crispus* is boiled in milk and strained, before sugar and other flavourings such as vanilla, cinnamon, brandy, or whisky are added. The end-product is a kind of jelly similar to pannacotta, tapioca, or blancmange.

USES OF CARRAGEENAN:-

Desserts, ice cream, cream, milkshakes, salad dressings, sweetened condensed milks, and sauces: gel to increase viscosity Beer: clarifier to remove haze-causing proteins Pâtés and processed meats (ham, e.g.): substitute for fat, increase water retention, and increase volume, or improve sliceability Toothpaste: stabilizer to prevent constituents separating Fruit Gushers: ingredient in the encapsulated gel Fire fighting foam: thickener to cause foam to become sticky shampoo and cosmetic creams: thickener, air freshener gels Marbling: the ancient art of paper and fabric marbling uses a carrageenan mixture on which to float paints or inks; the paper or fabric is then laid on it, absorbing the colours Shoe polish: gel to increase viscosity Biotechnology: gel to immobilize cells/enzymes Pharmaceuticals: used as an inactive excipient in pills/tablets Soy milk and other plant milks: used to thicken, in an attempt to emulate the consistency of whole milk

Diet sodas: to enhance texture and suspend flavours Pet food Personal lubricants Vegetarian hot dogs.

MATERIALS AND METHODS

MATERIALS REQUIRED :-

- PVA
- Carrageenan
- Glutaraldehyde
- Ethanol
- Hydrochloric acid
- Ciprofloxacin
- Nutrient agar
- Nutrient broth

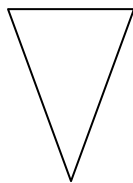
PREPARATION OF HYDROGEL :-

For the preparation of Hydrogels we have used 2% carrageenan and 10% PVA

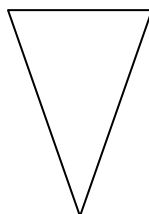


Carrageenan (2%) + PVA(10%)

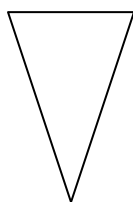
Aqueous solution of 10% PVA and 2% Carrageenan was prepared.



Different formulations were prepared by taking PVA and carrageenan in different proportions.



Blending of PVA and carrageenan was done for 10 mins at 120 rpm.



After blending of both the solutions these were poured into the moulds for the preparation of Hydrogels.

PREPARATION OF CROSS LINKER:-

Cross linkers are the polymers that links one polymer chain to the another. This polymers can be covalent or it can be ionic bond. Polymers can be of several types whether it can be a synthetic polymer or it can be natural polymer.

For the preparation of cross linker we have used Glutaraldehyde (50%) 10 ml , HCL (Hydrochloric acid) 1ml i.e 0.1 N HCL , and then we have added Ethanol 10ml to it like this the cross linker was prepare for the formation of the gel.

CHARACTERISATION:-

PHYSIOCHEMICAL CHARACTERISATION:-

- Microscopy
- SEM
- XRD
- FTIR
- Electrical
- Mechanical

OTHER CHARACTERISATION:-

- Hemocompatibility
- Swelling
- Drug release
- Antimicrobial
- MTT Assay

PHYSIOCHEMICAL CHARACTERIZATION:-

MICROSCOPY OR BRIGHT FIELD MICROSCOPY:-

Microscopy is also known as bright field microscopy. It is most simple of all optical microscopy . Sample illumination is transmitted and white light and the contrast of the sample is caused by the absorbance of some transmitted light in the dense areas of the sample . The light path of the bright field microscopy is very simple and there are no additional components which are required for the normal light setup. Microscope stand has a halogen lamp which transilluminate light source. It contains a condenser lens which focuses the light from the light source onto the sample. And lastly for viewing the image it has oculars.



Fig 1 . picture of bright field microscopy

SEM (Scanning Electron Microscopy):-

SEM analysis is also known as SEM microscopy. It is used effectively in microanalysis failure analysis of the solid samples. SEM is performed at high magnifications, and it generates high-resolution images and precisely measures very small features and objects . The SEM equipment includes a variable pressure system capable of holding wet and/or non-conductive samples with minimal preparation. The large sample chamber allows for the examination of samples up to 200 mm (7.87 in.) in diameter and 80 mm (3.14 in.) in height. High-resolution images are produced during SEM analysis at magnifications from 5x to 300,000x.



Fig 2 photograph of SEM

XRD (X-Ray Diffraction):-

X-ray powder diffraction (XRD) is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions. The analyzed material is finely ground, homogenized, and average bulk composition is determined. X-ray diffractometers consist of three basic elements: an X-ray tube, a sample holder, and an X-ray detector. X-rays are generated in a cathode ray tube by heating a filament to produce electrons, accelerating the electrons toward a target by applying a voltage, and bombarding the target material with electrons. When electrons have sufficient energy to dislodge inner shell electrons of the target material, characteristic X-ray spectra are produced. These spectra consist of several components, the most common being $K\alpha$ and $K\beta$. $K\alpha$ consists, in part, of $K\alpha_1$ and $K\alpha_2$. $K\alpha_1$ has a slightly shorter wavelength and twice the intensity as $K\alpha_2$. The specific wavelengths are characteristic of the target material (Cu, Fe, Mo, Cr). Filtering, by foils or

crystal monochrometers, is required to produce monochromatic X-rays needed for diffraction. $K\alpha_1$ and $K\alpha_2$ are sufficiently close in wavelength such that a weighted average of the two is used. Copper is the most common target material for single-crystal diffraction, with $\text{Cu}K\alpha$ radiation = 1.5418\AA . These X-rays are collimated and directed onto the sample. As the sample and detector are rotated, the intensity of the reflected X-rays is recorded. When the geometry of the incident X-rays impinging the sample satisfies the Bragg Equation, constructive interference occurs and a peak in intensity occurs. A detector records and processes this X-ray signal and converts the signal to a count rate which is then output to a device such as a printer or computer monitor. When the geometry of the incident X-rays impinging the sample satisfies the Bragg Equation, constructive interference occurs and a peak in intensity occurs. A detector records and processes this X-ray signal and converts the signal to a count rate which is then output to a device such as a printer or computer monitor. The geometry of an X-ray diffractometer is such that the sample rotates in the path of the collimated X-ray beam at an angle θ while the X-ray detector is mounted on an arm to collect the diffracted X-rays and rotates at an angle of 2θ . The instrument used to maintain the angle and rotate the sample is termed a goniometer. For typical powder patterns, data is collected at 2θ from $\sim 5^\circ$ to 70° , angles that are preset in the X-ray scan.



Fig 3 photo of XRD machine

FTIR (FOURIER TRANSFORM INFRARED SPECTROSCOPY):-

Fourier transform infrared spectroscopy (FTIR)[1] is a technique which is used to produce an infrared spectrum of absorption, emission, photoconductivity or Raman scattering of a solid, liquid or gas. An FTIR spectrometer simultaneously collects high spectral resolution data over a wide spectral range. This confers a significant advantage over a dispersive spectrometer which measures intensity over a narrow range of wavelengths at a time. The term Fourier transform infrared spectroscopy originates from the fact that a Fourier transform is required to convert the raw data into the actual spectrum.

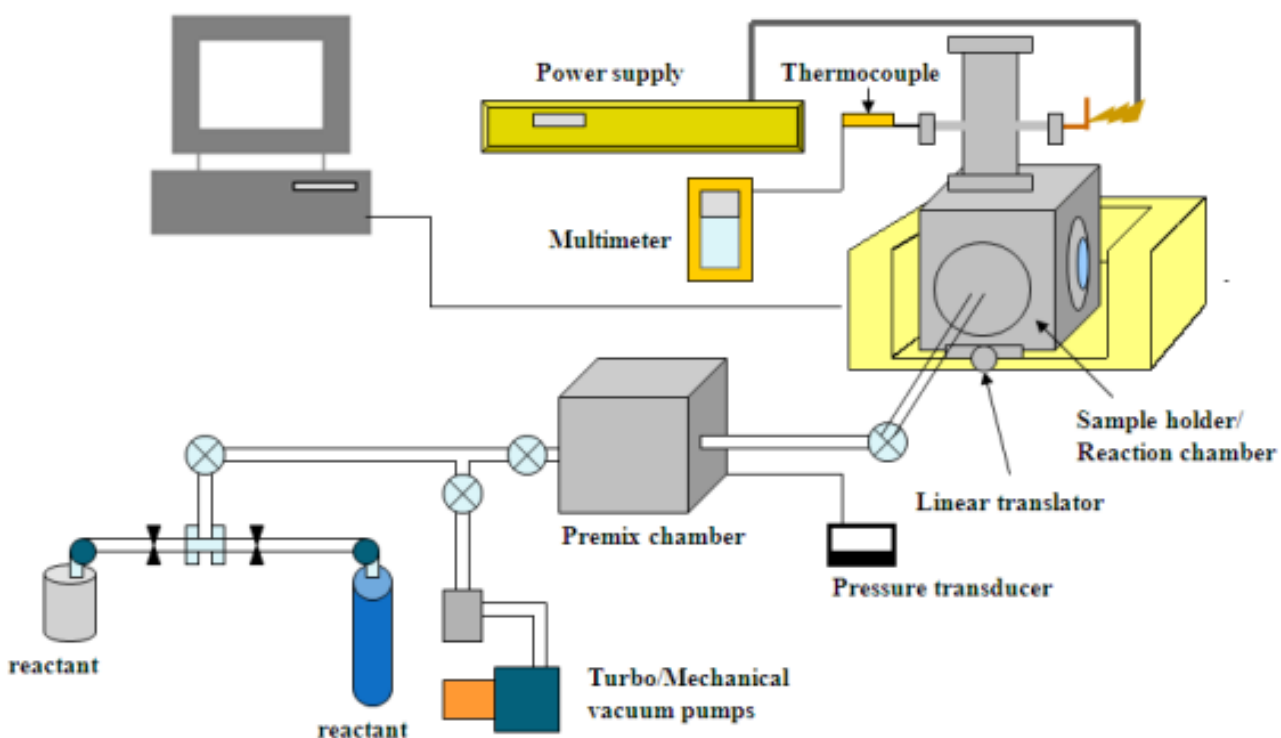


Fig 4 showing the working principle of FTIR

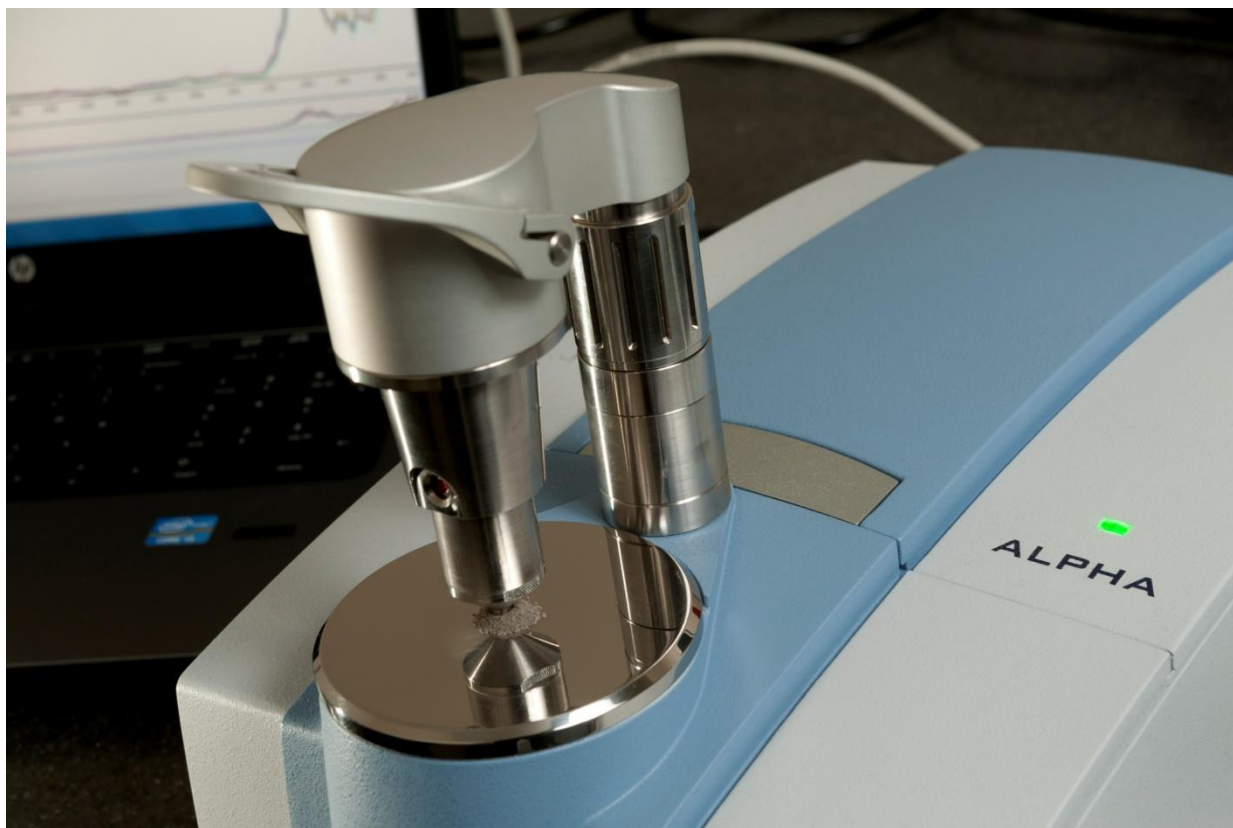


Fig 5 picture showing the photograph of FTIR

ELECTRICAL ANALYSIS:-



FIG SHOWING ELECTRICAL ANALYSIS SET UP

MECHANICAL ANALYSIS:-

Mechanical testing provides information about the suitability of a material for its intended application to help companies design reliable products that will perform as expected. Mechanical testing services measure materials under various temperature, tension, compression and load conditions to determine:

Strength

Hardness

Ductility

Impact resistance

Fracture toughness

Elongation

Stress

Mechanical Test Ranges

Elevated temperature tensile testing Tensile testing of metal parts and specimens in all sizes, from the smallest fasteners to huge tubing and bolts on our 600,000 lb. capacity machines. A 10,000 lb. capacity tensile machine provides plastics testing Elevated temperature tensile testing using a furnace carousel to process up to three samples at once. Samples can be heated to 1800°F Stress rupture and creep testing comply with ASTM standards and can be performed at temperatures up to 2000°F Fracture toughness and fatigue testing with equipment that can generate up to 55,000 lbs. of tensile or compressive force and controls the test temperature between -250°F and +400°F Rockwell, Brinell & Superficial hardness testing is available for metals, while a Shore Durometer hardness tester provides plastics testing Charpy impact testing is performed from -452°F to 500°F.

OTHER CHARACTERISTICS:-

HEMOCOMPATIBILITY TEST:-

Hemocompatibility test means to find out whether the components are hemocompatible or not. For hemolysis study Fresh goat blood, collected in a beaker, containing Sodium Citrate (3.8gm%, 10:1) was diluted with normal saline solution (8ml blood+10ml normal saline). For checking hemolysis, 0.2ml of the diluted blood was added to 10ml of 0.1% Sodium Carbonate solution and optical density measured at 545nm in a UV-spectrophotometer. Take a 5mmX5mm sample without sharp edges in a standard tube containing 10ml of normal saline kept in an incubator at 37 °C for 30min providing temperature equilibration. Add 0.2ml of the diluted

blood to the test-tube, mix gently and incubate for 60min. For positive control, 0.2ml of diluted blood was taken in 10ml of 0.1% Sodium Carbonate solution and for negative control, 0.2ml of diluted blood was taken in 10ml of normal saline solution and incubated for 60min at 37°C. In a similar manner, material sample was incubated for 60min at 37°C. After 60mins of incubation, all the test-tubes were centrifuged for 5mins at 3000rpm and the supernatant was carefully removed and transferred to the cuvette for spectroscopic analysis at 545nm wavelength and percentage hemolysis was calculated. $\text{Percentage hemolysis} = \frac{[\text{OD}(\text{test}) - \text{OD}(\text{negative control})] \times 100}{[\text{OD}(\text{positive control}) - \text{OD}(\text{negative control})]}$

Where, OD= optical density at 545nm Percentage hemolysis was calculated based on average of two replicates.

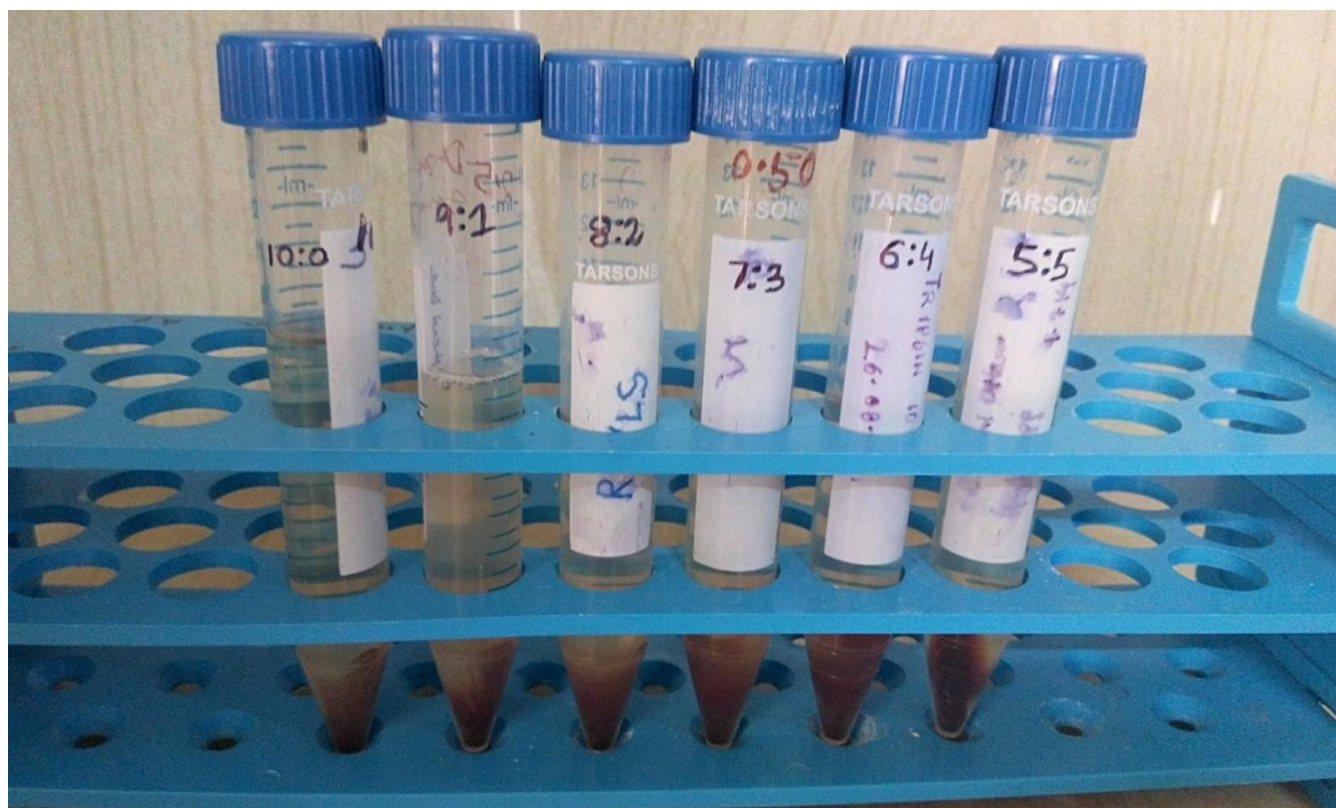


Fig 6 Picture showing tubes showing hemolysis

SWELLING STUDIES :-

Swelling studies were done to check the water absorbing capacity of the Hydrogels.

- Firstly a thin slice of hydrogel was cut weighing 0.5gm .
- Then initial weight was measured.
- Then it was poured into the PBS solution of pH 7.4

- First within 15 mins the weight of the samples were checked .
- Then within 5 hrs at regular interval thw weight was measured till the weight remain constant.

DRUG RELEASE STUDIES:-

Drug Release studies were done to see the control drug delivery of the system .This study was carried out for 48 hours . 6 liters of PBS were prepared .

COMPOSITION OF PBS:-

Nacl -8gm

Kcl -0.2gm

Na₂ HPO₄-1.44gm

KH₂PO₄ – 0.24gm

A dialysis membrane was attached to the testtube and a piece of drig loaded sample was inserted inside the testtube and under that beaker was kept containg the PBS solution .

First intial time was 15 mns for 1hr then for 30 mins then for 1hr gradually the experiment was carried out.



Fig set up for drug release studies

ANTIMICROBIAL ACTIVITY :-

An antimicrobial is an agent that kills microorganisms or inhibits their growth.[1] Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. For example, antibacterials are used against bacteria and antifungals are used against fungi. They can also be classified according to their function. Agents that kill microbes are called microbicidal, while those that merely inhibit their growth are called microbiostatic. The use of antimicrobial medicines to treat infection is known as antimicrobial chemotherapy, while the use of antimicrobial medicines to prevent infection is known as antimicrobial prophylaxis.

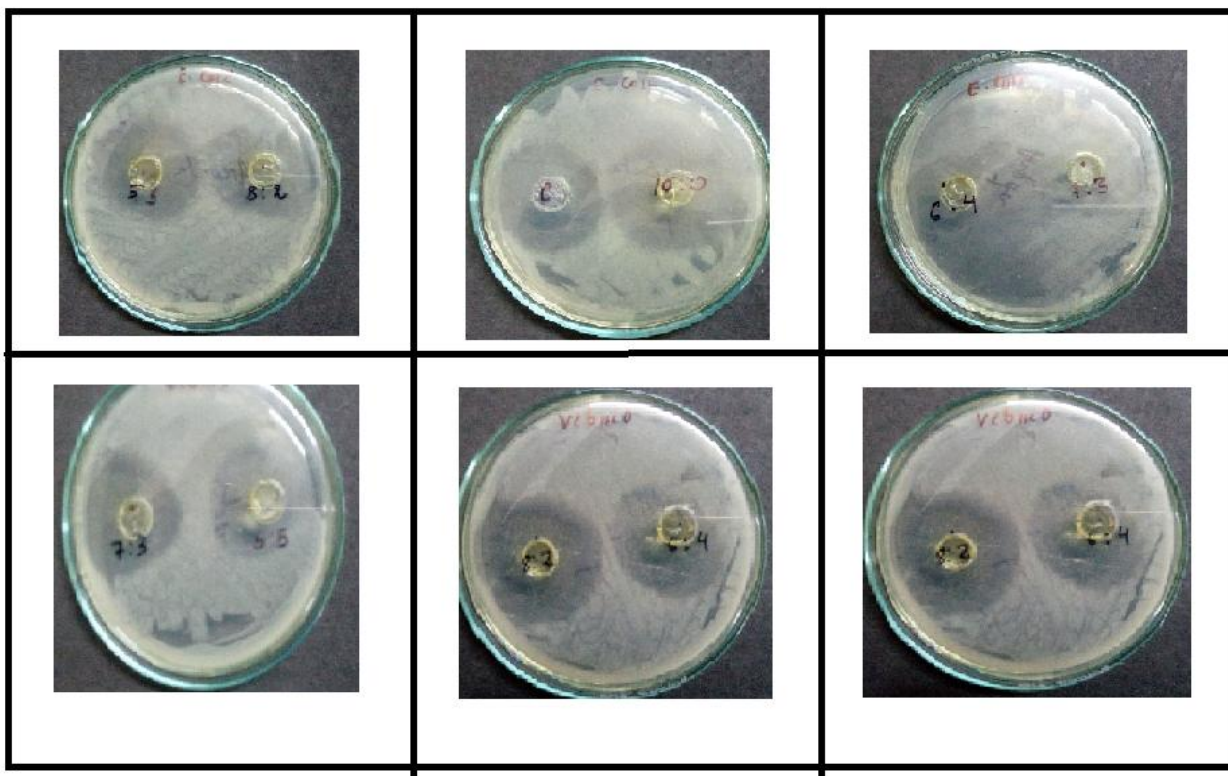


Fig 7 showing the Antimicrobial activity

MTT ASSAY :-

MTT Assay was done to check the cell viability of hydrogel compounds.

The MTT assay is a colorimetric assay for assessing cell viability. NAD(P)H-dependent cellular oxidoreductase enzymes may, under defined conditions, reflect the number of viable cells present. These enzymes are capable of reducing the tetrazolium dye MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to its insoluble formazan, which has a purple color. Other closely related tetrazolium dyes including XTT, MTS and the WSTs, are used in conjunction with the intermediate electron acceptor, 1-methoxy phenazine methosulfate (PMS). With WST-1, which is cell-impermeable, reduction occurs outside the cell via plasma membrane electron transport.[1] Tetrazolium dye assays can also be used to measure cytotoxicity (loss of viable cells) or cytostatic activity (shift from proliferation to quiescence) of potential medicinal agents and toxic materials. MTT assays are usually done in the dark since the MTT reagent is sensitive to light.

In this process :

- Leachets were prepared by putting the Hydrogels samples inside the PBS for 24 hrs
- Then after 24 hrs the leachets were collected.
- First day media was added
- 2nd day media was removed fresh media was added and to it leachets
- Next day leachets were removed and mtt was added and incubated for 4hrs and after that DMSO was added and reading was taken at 562 nm.

RESULTS AND DISCUSSION

PHOTOGRAPHS OF PREPARED GELS :-

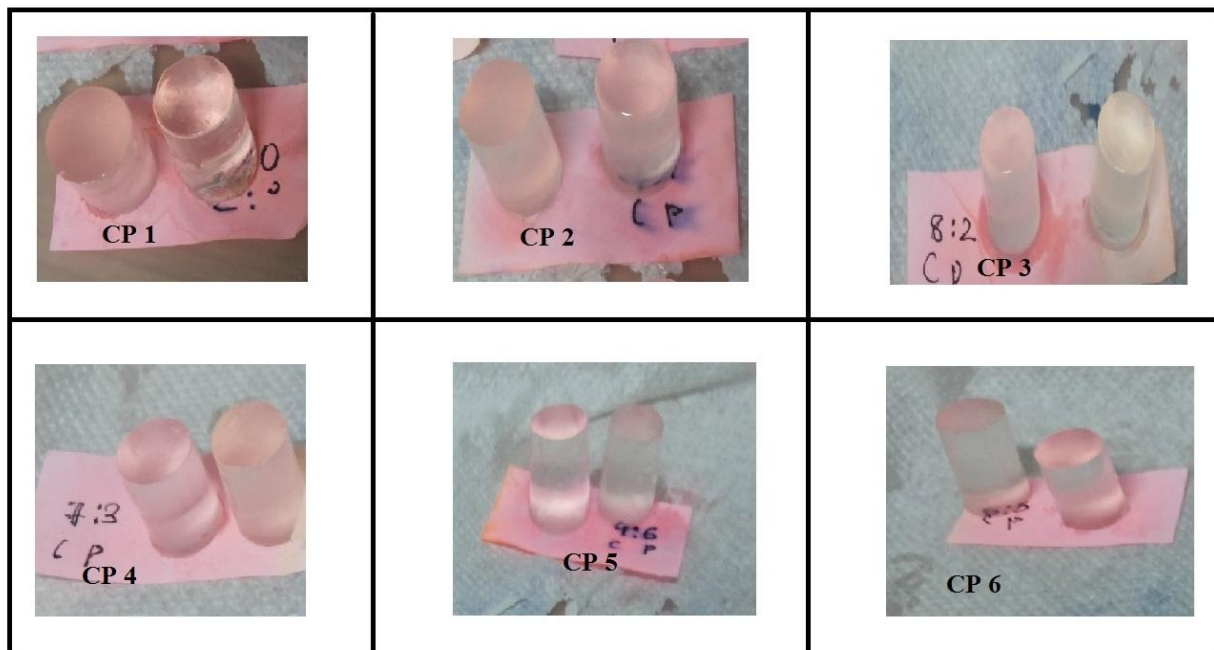


Fig 9 pictures of prepared hydrogels

COMPOSITION OF HYDROGELS

SL.NO	SAMPLE CODE	CARRAGEENAN	PVA
1	CP1	10	0
2	CP2	9	1
3	CP3	8	2
4	CP4	7	3
5	CP5	6	4
6	CP6	5	5

PHOTOGRAPHS OF MICROSCOPY:-

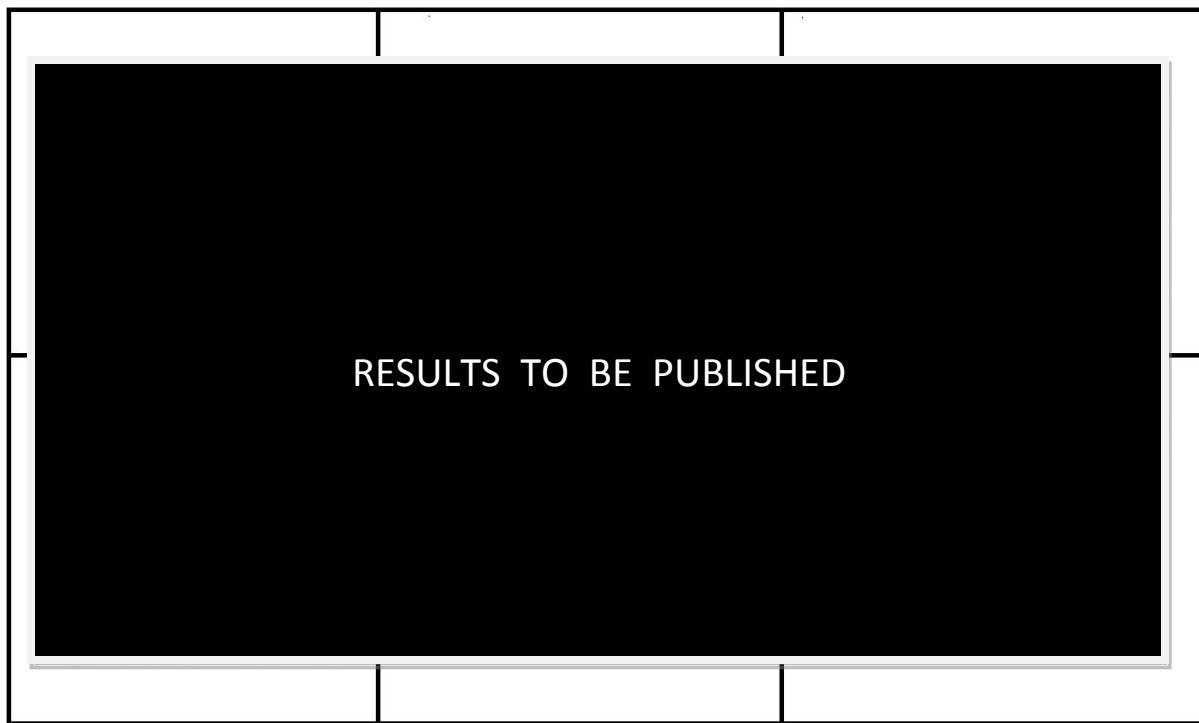


Fig 10 microscopy pictures

This microscopy of PVA and Carrageenan were the phase separated system.

Where PVA shows the dispersion phase and carrageenan dispersve phase.

PHOTOGRAPHS OF SEM:-

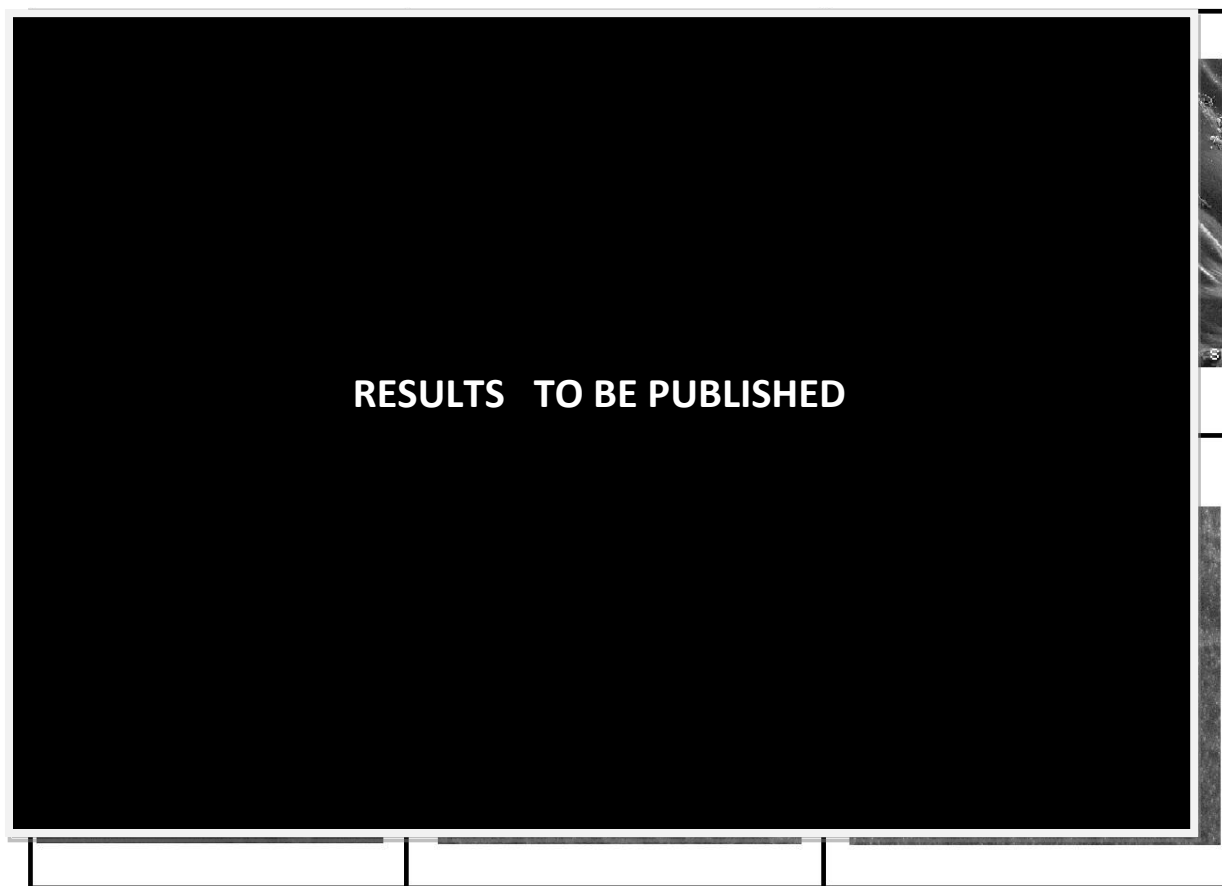
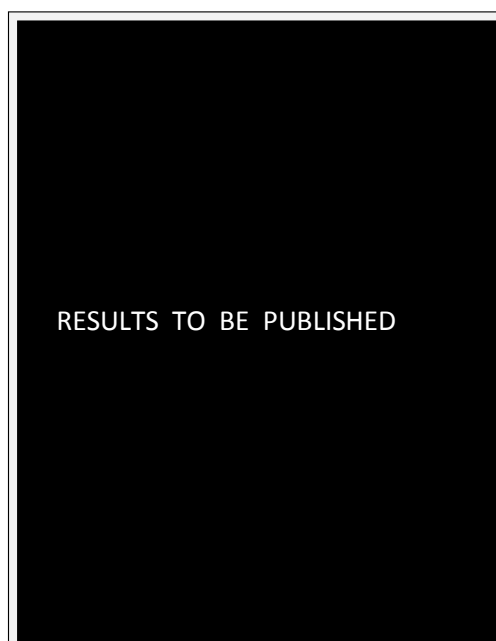


Fig 11 photos of SEM

XRD (X-Ray diffraction)

Fig 12 XRD GRAPHS



FTIR GRAPHS :-

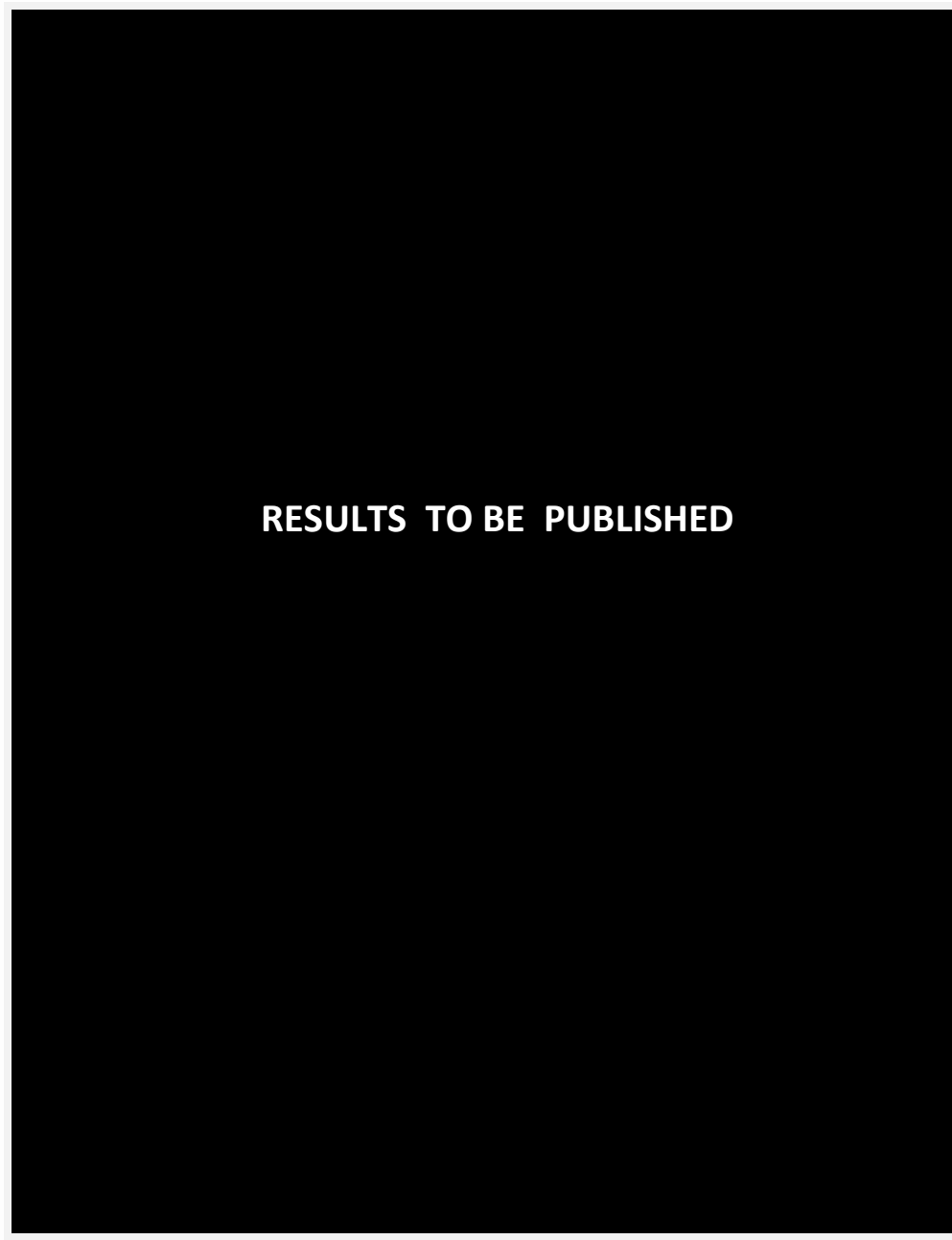


Fig 13 showing the FTIR Graph

ELECTRICAL ANALYSIS :-



Fig 14 showing the electrical analysis of Impedance and frequency.

MECHANICAL ANALYSIS :-

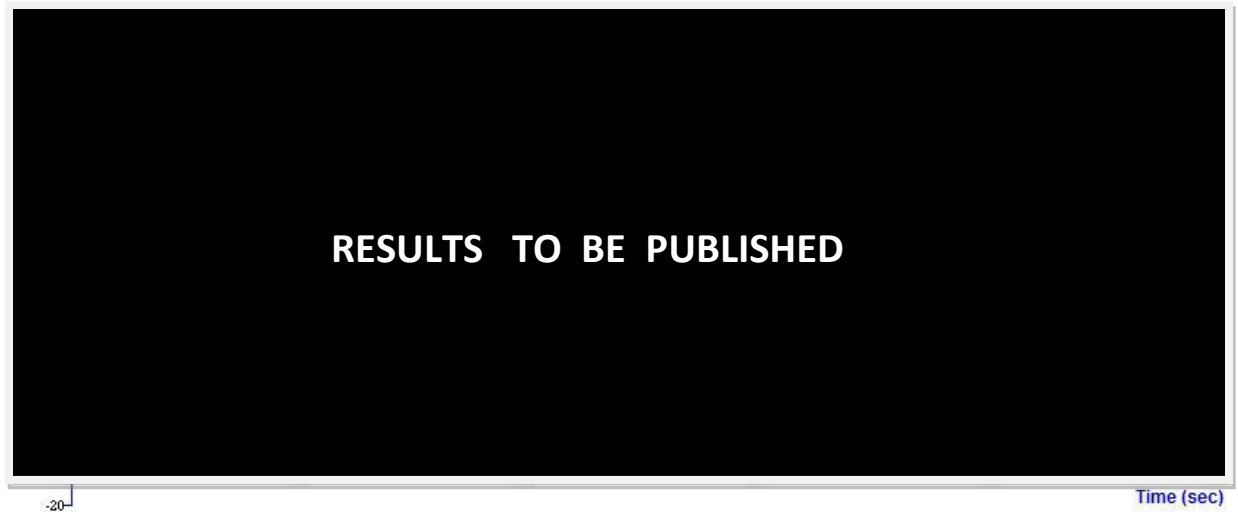


Fig 15 showing the mechanical analysis

ANTIMICROBIAL GRAPHS :-

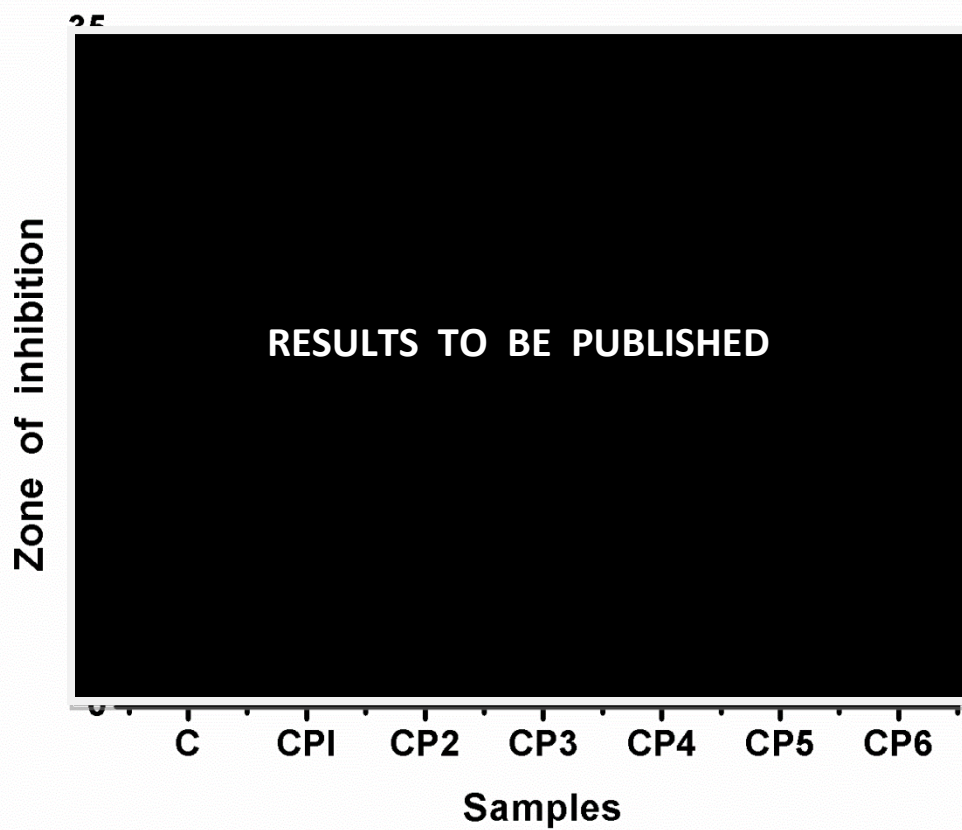
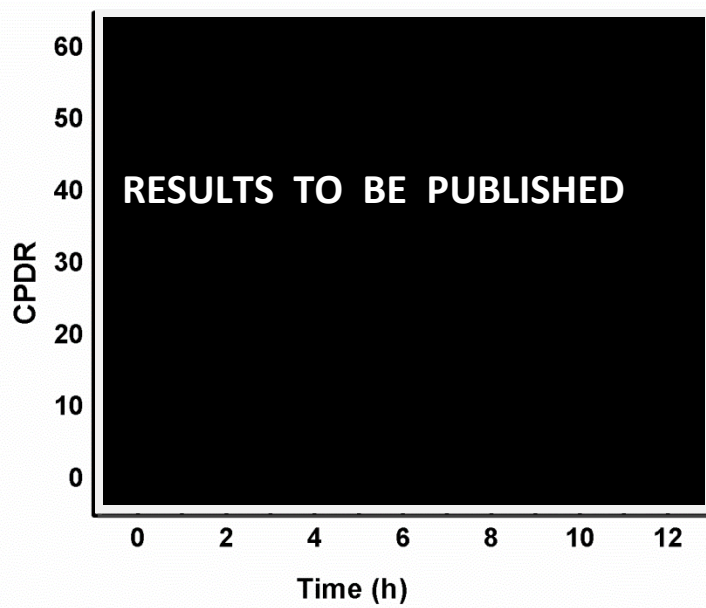
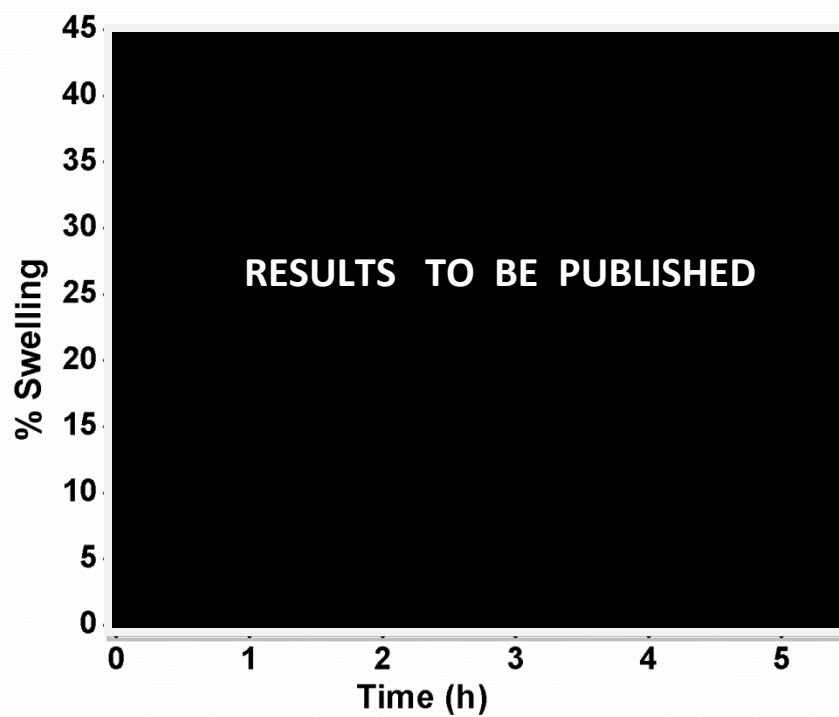


Fig 16 showing the antimicrobial activity.

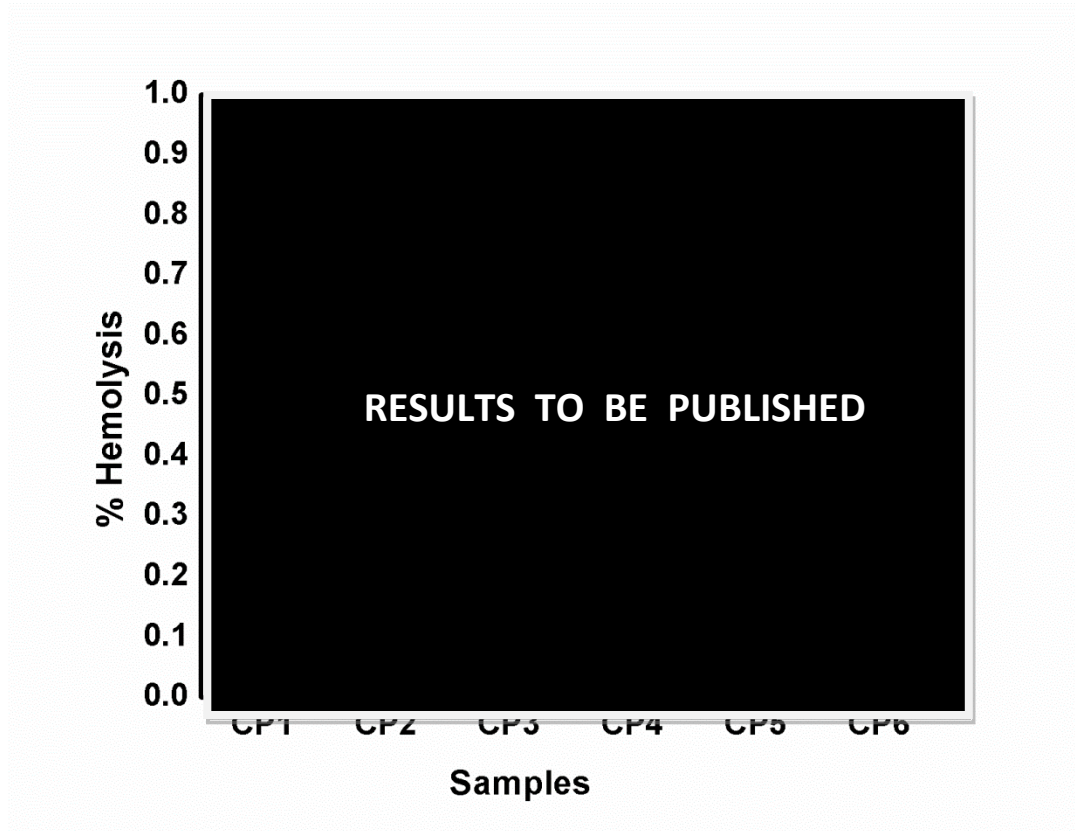
DRUG RELEASE STUDIES :-



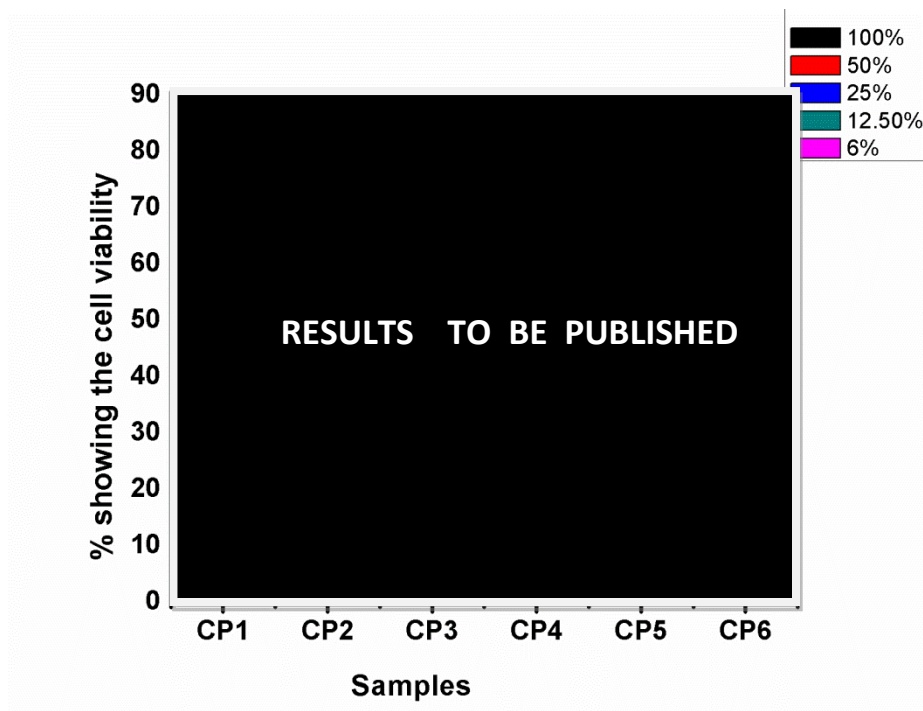
SWELLING STUDIES :-



HEMOCOMPATIBILITY TEST:-



MTT ASSAY :-



CONCLUSION

The above characterisation of the hydrogels showed that the hydrogels can be used for a control drug delivery system. It can be used for biomedical applications. We found that CP 6 shows the better result as comparable to the rest of the concentrations as it shows the high hemocompatibility and antimicrobial activity. It also shows good swelling ability and uniform drug release profile

REFERENCES

1. Couldwell WT, Chen TC, Weiss MH, et al: Cranioplasty with the Medpor porous polyethylene Flexblockimplant. Technical note. J Neurosurg 81:483–486, 1994
2. Larry M. Wolford, and Rogerio Z. Freitas. Porous block hydroxyapatite as a bone graft substitute in the correction of jaw and craniofacial deformities. BUMC Proceedings 1999;12:243-246.
3. Cottrell DA, Wolford LM. Long-term evaluation of the use of coralline hydroxyapatite in orthognathic surgery. J Oral Maxillofac Surg 1998;56:935.
4. Gonzalez-Rodriguez ML, Perez-Martinez JI, Merino S, Fini A, Rabasco AM. Drug Dev. IndPharm. 2001 May;27(5):439-46. Channeling agent and drug release from a central core matrix tablet. Departamentode Farmacia y Tecnologia Farmaceutica, Universidad de Sevilla, Spain.
5. Dembczynski R., Jankowski T. Determination of pore diameter and molecular weight cut-off of hydrogel-membrane liquid-core capsules for immunoisolation. Journal of Biomaterials Science, Polymer Edition, 1October 2001, vol. 12, no., pp. 1051-1058(8)
6. <http://landau1.phys.virginia.edu/classes/241L/poise/poise.htm>
7. Stein, H.L., Ticona, P.E., .Ultra High Molecular Weight Polyethylene (UHMWPE), Guide to Engineering Plastic Families: Thermoplastic Resins, Vol. 2. Engineered Materials Handbook, ASM International, Materials Park, OH, pp. 167-171, 1999.
8. Callister Jr., W. D. 2003. Materials Science and Engineering—An Introduction , 6th ed. New York: Wiley, Sections 6.1–6.12, 8.1–8.4.
9. Radhakrishnan S. and Khedkar S. P., Application of dip-coating process for depositing conducting polypyrrole films. Thin Solid Films, Volume 303, Issues 1-2, 15 July 1997, Pages 167-172.
10. Yoshikazu Miyake, Yasuhiro Sekiguchi and Shinzo Kohjiya. Formation of percolated structure during solvent casting of polymer blend-solvent systems. Journal of Chemical Engineering of Japan, vol 26 no 5 pp 543-550(1993).
11. Bennet J.M. & Mattson Lars “Introduction to Surface Roughness and Scattering”, Optical Society of America, Washington D.C. 1989.
12. Ravaglioli and Krajewski, Bioceramics, Published by Chapman & Hall, 3 rd edition; page: 100-101, 1991..

13. Peppas, A. N. and Merrill, W. E. J. Biomed. Mater. Res. 1977, 11, 423
14. Watase, M., Nishinary, K. and Nambu, M. Polym. Commun. 1983, 24, 52
15. Hyon, S. H., Cha, W. I. and Ikada, Y. Polym. Bull. 1989, 22, 1199 Ofstead, R. F. and Poser, C. I. Polym. in Aqueous Media 1989, 223, 61
16. Peppas, N. A. 'Hydrogels Medicine and Pharmacy' (Ed. N. A. Peppas), CRC Press, Boca Raton, 1987, Vol. 2, p. 1
17. Watase, M. and Nishinary, K. Makromol. Chem. 1988, 189, 87112 Nagura, M., Hamano, T. and Ishikawa, H. Polymer 1989, 30, 762
18. Yamaura, K., Itoh, M., Tanigami, T. and Matsuzawa, S. J. Appl. Polym. Sci. 1989, 37, 2709
19. Urushizaki, F., Yamaguchi, H., Nakamura, K., Numajiri, S., Sugibayashi, K. and Morimoto, Y. Int. J. Pharm. 1990, 58, 135
20. Lozinski, V. I., Vainerman, E. S., Domotenko, L. V., Mamtsis, A. M., Titova, E. F., Belavtseva, E. M. and Rogozhin, S. V. Colloid Polym. Sci. 1986, 264, 19
21. Andrade, J., King, R. and Gregonis, D. in 'Hydrogels for Medical and Related Applications' (Ed. J. Andrade), ACS Symp. Ser., American Chemical Society, Washington, DC, 1976, p. 206
22. Miller, D. and Peppas, N. Biomaterials 1986, 7, 329
23. Blank, Z. and Reimschuessel, A. J. Mater. Sci 1974, 9, 1815
24. Trieu, H. and Qutubuddin, S. Colloid Polym. Sci. 1994, 272, 301
25. Box, E. P. B. and Draper, R. N. in 'Empirical Model-Building and Response Surfaces', John Wiley & Sons, New York, 1987
26. 'Standard Test Method for Rubber Property – Durometer Hardness', ASTM D2240-86, in 'Annual Book of ASTM Standards' Vol. 9.01, American Society for Testing and Materials, Philadelphia, PA
27. 'Standard Test Method for Rubber Properties in Tension', ASTM D412-87, in 'Annual Book of ASTM Standards' Vol. 9.01, American Society for Testing and Materials, Philadelphia, PA
28. 'Standard Test Method for Rubber Property - Tear Resistance', ASTM D624-86, in 'Annual Book of ASTM Standards' Vol. 9.01, American Society for Testing and Materials, Philadelphia, PA
29. Komatsu, M., Inoue, T. and Miyasaka, K. J. Polym. Sci., Polym. Phys. Edn 1986, 24, 303
30. Tong, H. M., Noda, I. and Gryte, C. C. Colloid Polym. Sci. 1984, 262, 589

- 31 Yokoyama, F., Masada, I., Shimamura, K., Ikawa, T. and Monobe, K. Colloid Polym. Sci. 1986, 264, 595
- 32 Szmant, H. H. in 'Dimethyl Sulfoxide' (Eds S. W. Jacob, E. E. Rosenbaum and D. C. Wood), Marcel Dekker, New York, 1971, Vol. 1
- 33 Rasmussen, D. H. and MacKenzie, A. P. Nature 1968, 220, 1315
- 34 Fox, M. F. and Whittingham, K. P. J. Chem. Soc., Faraday Trans. I 1975, 71, 1407
- 35 Bowen, D. E., Priesand, M. A. and Eastman, M. P. J. Phys. Chem. 1974, 78, 261131
- Callister, S., Keller, A. and Hikmet, R. M. Makromol. Chem., Macromol. Symp. 1990, 39, 1932
- Ohkura, M., Kanaya, T. and Kaji, K. Polymer 19